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Synonymy of two species of *Bipolaris* from aquatic crops of *Poaceae*

Zi-Lan Xiao[1], Kevin D. Hyde[2], & Jing-Ze Zhang[1*]

[1]*Institute of Biotechnology, College of Agriculture & Biotechnology, Zhejiang University, Hangzhou 310058 China*

[2]*Institute of Excellence in Fungal Research, School of Science, Mae Fah Luang University, Chiang Rai 57100 Thailand*

*Correspondence to: *jzzhang@zju.edu.cn*

Abstract— Our morphological data indicate that conidia of *Bipolaris zizaniae* (a pathogen causing brown spot on leaves of *Zizania latifolia*) are similar to those of *B. oryzae*. Our sequences of the rDNA ITS, GPDH, and EF1-α gene regions from *B. zizaniae* and blast searches revealed a 99–100% similarity with sequences of *B. oryzae*. Phylogenetic studies also cluster *B. zizaniae* isolates with *B. oryzae* in a clade with 100% bootstrap support. Pathogenicity testing also confirmed that *B. zizaniae* does infect both *Zizania latifolia* and rice, causing brown spots.

Key words—graminicolous fungi, molecular phylogeny, taxonomy

Introduction

Zizania latifolia (Griseb). Turcz. ex Stapf is a perennial grass parasitized by the smut fungus *Ustilago esculenta* Henn., which causes swelling of its upper culms. The swollen culms are used as an aquatic vegetable, commonly called jiaobai (white bamboo) in China (Zhang et al. 2012). Due to its unique flavor and delicacy, the demand for jiaobai has steadily increased in tandem with the rapid economic development of China. Zhejiang Province has become the largest *Z. latifolia* cultivation area in China (Xu et al. 2009). Brown spot is one of the most severe leaf spot diseases of this crop plant. With large-scale cultivation and changed growing conditions, brown spot incidence has become more severe in the fields, and the disease has become a severe problem in crop production. The pathogen causes a large number and areas of coalescing spots (especially during seedling production in greenhouses), and these spots expand or coalesce to form large lesions, eventually resulting withering and death of entire leaves (Fig. 1A–D).

The pathogen causing this disease was first described as *Helminthosporium zizaniae* by Nisikado (1929). Although *H. zizaniae* resembles *H. oryzae* morphologically and by infecting *Oryza sativa* L. [rice], Nisikado (1929) differentiated the two species based on the shape of the conidial base, conidial dimensions, and conidial hilum. Shoemaker (1959) transferred *H. zizaniae* and *H. oryzae* to *Bipolaris,* a placement later accepted by Shoemaker (1966) and Sivanesan (1987). Sivanesan (1987) used conidial dimensions as an important character in his dichotomous key to *Bipolaris;* he distinguished *B. zizaniae* as having >150 µm long conidia compared with the <150 µm long conidia in *B. oryzae*. However, conidial dimensions are usually variable in *Bipolaris* (Sivanesan et al. 1987; Manamgoda et al. 2012), and the conidial length in *H. oryzae* is not stable and can be more than 150 µm in some strains (Chang 1978). Other studies showed that *B. oryzae* is interfertile with *B. zizaniae* and that their isolates from rice and *Zizania* are reciprocally infective (Chang 1974; Tsuda & Ueyama 1975, 1976). *Bipolaris zizaniae* and *B. oryzae* are now regarded as conspecific (Manamgoda et al. 2014).

Accurate identification and precise naming of species are crucial, for species names provide an ideal framework for storage and information retrieval for effective disease management (Inderbitzin et al. 2011, Manamgoda et al. 2012). *Bipolaris zizaniae* is an important pathogen on *Z. latifolia*, but due to limited distribution, there is little information on its effects; even the morphological descriptions of the pathogen are inadequate (Nisikado 1929, Sivanesan 1987). In contrast, *B. oryzae* [sexual state previously known as *Cochliobolus miyabeanus*] causes brown spots on leaves and glumes of rice, leading to serious losses in yield (Scheffer 1997). Thus this pathogen and rice disease have been studied worldwide (Kulkarni et al. 1980, Kubo et al. 1989, Amadioha 2002, Manandhar et al. 1998, Xio et al. 1991). Nonetheless, the relationship between *B. zizaniae* and *B. oryzae* is unresolved. With the use of molecular taxonomy, DNA sequence analyses allow testing the morphological species circumscriptions and providing new insights into species relationships, especially for complexes of closely related, morphologically similar species (Manamgoda et al. 2012, 2014; Udayanga et al. 2012; Hyde et al. 2014). Studies of *Bipolaris* species have already revealed relationships between morphological species circumscriptions and molecular data, but there has been no study of *B. zizaniae* (Manamgoda et al. 2011, 2012, 2014; Hyde et al. 2014).

We used sequences from the ITS (internal transcribed spacer), GPDH (glyceraldehyde-3-phosphate dehydrogenase), and EF1-α (translation elongation factor 1-α) gene regions to investigate the phylogenetic relationships of six *Bipolaris* cultures isolated from *Zizania latifolia* in southeast China. The objectives were to review morphological similarities in *B. zizaniae* and *B. oryzae* and to resolve their relationship.

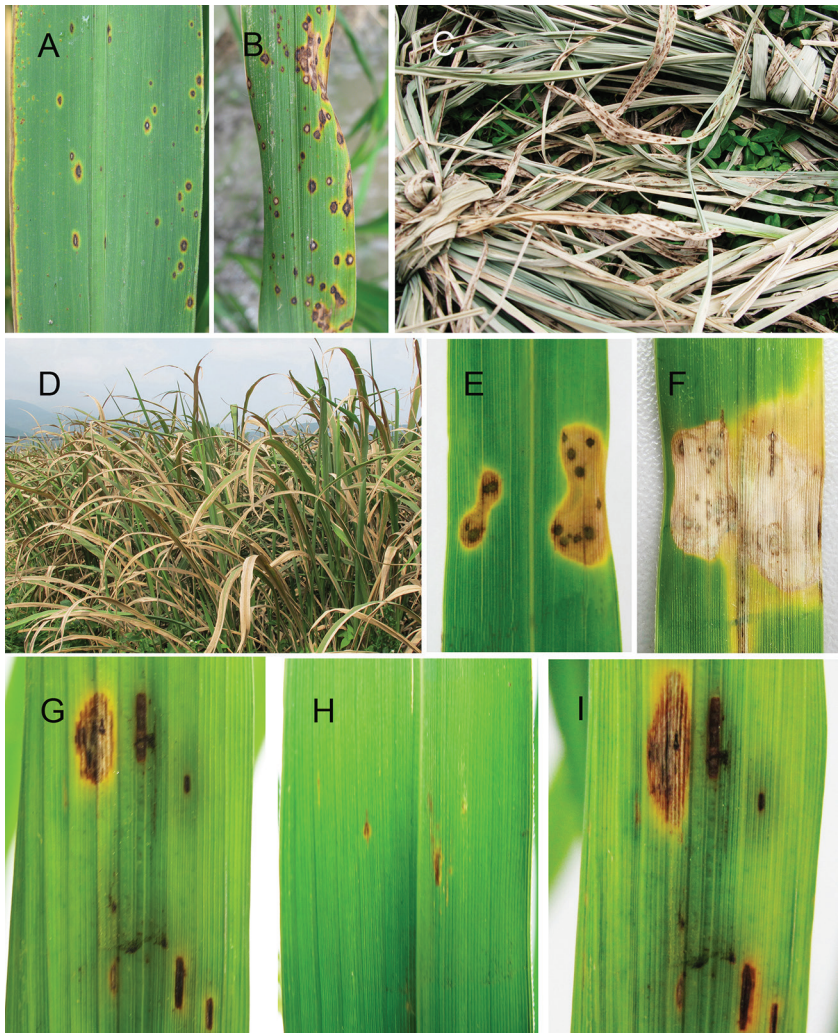

Figure 1 Symptoms of *Bipolaris* brown spot on *Zizania latifolia* and *Oryza sativa.* A–D. On nature host of *Z. latifolia*. A. Early spots. B. Severe spots. C. Removed leaves with severe brown spots from seedlings in the greenhouse. D. Leaf blight. E–F. Symptom variability on backs of leaves of *Z. latifolia* after artificial inoculation. E. Five days after inoculation. F. Eighteen days after inoculation. G–I. Symptom variability on leaves of *O. sativa* after artificial inoculation. G. Brown spots eight days after inoculation. H. Brown spots on rear of leaf nine days after inoculation. I. Brown spots twelve days after inoculation.

Table 1. *Bipolaris* and *Curvularia* isolates used in multi-gene DNA sequence analysis.

Species	Isolate	Host	Location	GenBank No.		
				ITS	GPDH	EF1-α
B. coffeana	ICMP 6128	*Cynodon dactylon*	New Zealand	JX256412	JX276427	JX266581
B. microlaenae	CBS 280.91 *	*Microlaena stipoides*	Australia	JN601032	JN600974	JN601017
	BRIP 15613			JN192378	JN600973	JN601017
B. nodulosa	CBS 160.58			JN601033	JN600975	JN601019
B. oryzae	MFLUCC 10-0715 *	*Oryza sativa*	Thailand	JX256416	JX276430	JX266585
	MFLUCC 10-0694	*O. sativa*	Thailand	JX256413	JX276428	JX266582
	MFLUCC 10-0733	*O. sativa*	Thailand	JX256417	JX276431	JX266586
	MFLUCC 13-0511	***Zizania latifolia***	**China**	**KF688965**	**KF688971**	**KF688977**
	MFLUCC 13-0512	***Z. latifolia***	**China**	**KF688966**	**KF688972**	**KF688978**
	MFLUCC 13-0513	***Z. latifolia***	**China**	**KF688967**	**KF688973**	**KF688979**
	MFLUCC 13-0514	***Z. latifolia***	**China**	**KF688968**	**KF688974**	**KF688980**
	MFLUCC 13-0515	***Z. latifolia***	**China**	**KF688969**	**KF688975**	**KF688981**
	MFLUCC 13-0516	***Z. latifolia***	**China**	**KF688970**	**KF688976**	**KF688982**
B. peregianensis	BRIP 12790 *	*Cynodon dactylon*	Australia	JN601034	JN600977	JN601022
C. australiensis	CBS 172.57	*Oryza sativa*	Vietnam	JN601026	JN601036	JN601003
C. coicis	CBS 192.29 *	*Coix lacryma*	Japan	JN192373	JN600962	JN601006
C. ellisii	CBS 193.62		Pakistan	JN192375	JN600963	JN601007
C. graminicola	BRIP 23186a		Australia	JN192376	JN600964	JN601008
C. hawaiiensis	CBS 173.57 *	*Oryza sativa*	Hawaii, USA	JN601029	JN600966	JN601010
	BRIP 1097	*Chloris gayana*	Australia	JN601030	JN600967	JN601011
C. heteropogonis	CBS 284.91 *	*Heteropogon contortus*	Australia	JN192379	JN600969	JN601013
C. homomorpha	CBS 156.60			JN192380	JN600970	JN601014
C. lunata	CBS 730.96 *		USA	JX256429	JX276441	JX266596
	MFLUCC 10-0706	*Oryza sativa*	Thailand	JX256431	JX276443	JX266598
C. ovariicola	BRIP 15882			JN601031	JN600976	JN601015
C. ravenelii	BRIP 13165	*Sporobolus fertilis*	Australia	JN192386	JN600978	JN601024
C. spicifera	CBS 274.52		Spain	JN192387	JN600979	JN601023
C. tripogonis	BRIP 12375		Australia	JN192388	JN600980	JN601025
C. tuberculata	CBS 146.63 *	*Zea mays*	India	JX256433	JX276445	JX266599

* = ex-type cultures. New sequences are presented in bold font; other sequences are from Manamgoda et al. 2011, 2012, 2014.

Materials & methods

Collection and isolation of fungi

Diseased leaves of *Zizania latifolia* with brown spots growing in fields or greenhouses were collected and returned to the laboratory. Tissue pieces (each approximately 5 × 5 mm) were cut from the margins of brown spots, between healthy and infected parts of leaves, which were previously surface disinfected with 0.5% NaOCl. The pieces were plated on to PDA medium and incubated at 25°C for 7 days. Mycelia from the infected samples were transferred to fresh PDA medium. Single-spore isolation was performed as described by Chomnunti et al. (2014). Agar discs (with mycelium on the surface, each approximately 5 × 5 mm) from these isolates were stored in 1.5 mL cryotubes with 20% glycerol at –70°C.

Herbarium materials are deposited at MFLU herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU). The cultures are maintained at Mae Fah Luang University Culture Collection (MFLUCC) and Institute of Biotechnology, Zhejiang University, Zhejiang Province, China (ZJU).

DNA extraction

Genomic DNA was extracted using a modified protocol of Zhang and Li (2009). Fungal isolates were cultured on PDA at 25°C for 7 days. Fresh mycelia were scraped from the surfaces of PDA plate and grounded to a fine powder in liquid nitrogen. 500 mg power was transferred to a 1.5 mL microcentrifuge tube and 700 µL of preheated (60°C) 2× CTAB extraction buffer (2% hexadecyltri-methlammonium bromide(w/v), 100 mM Tris–HCl, 1.4 M NaCl, 30 mM EDTA, pH 8.0) was added. The solution was incubated in a water bath at 65°C for 60 min with a gentle swirling. Following a phenol/chloroform extraction, the genomic DNA was precipitated by isopropanol in the presence of sodium acetate. The precipitate was rinsed with 70% ethanol centrifuged at 12,000 rpm and genomic DNA was resuspended in 50 µL TE buffer and stored in –20°C.

PCR amplification

The DNA fragments were amplified in an automated thermal cycler (Eppendorf AG, Germany). Amplification was performed in a 50 µL reaction volume which contained 5 µL 10× PCR buffer, 1 µL of each primer (10 µM), 2 µL template DNA, and 0.5 µL Taq DNA polymerase. Primers ITS6 and ITS4 (Zhang & Li. 2009) were used to amplify the ITS and 5.8 S regions. The thermal cycling program was performed with 35 cycles after an initial denaturation at 95°C for 4 min. Each cycle included a denaturation step at 95°C for 1min, an annealing step at 55°C for 1min, and an extension step at 72°C for 1.5 min. The primers gpd1/ gpd2 (Berbee et al. 1999) were used for amplifying the GPDH gene with the changes of annealing temperature 52°C. Similarly, the EF-1α gene was amplified using primers EF 983/2218R (Schoch et al. 2009) with the changes of annealing temperature 54°C.

Sequence alignment and phylogenetic analysis

The purified PCR products were submitted to Sunny Biotechnology Company Limited (Shanghai, China) for sequencing in both directions. Sequence files were assembled and edited using BioEdit software (Hall 1999). A blast search was performed

for searching the highly similar sequences in the GenBank database and related sequences were downloaded (Table 1). The sequences were aligned with Clustal X 25 (Thompson et al. 1997). Sequences were analyzed phylogenetically in PAUP v4.0b10 (Swofford 2002) and trees were visualized with TreeView (Page 1996). Phylogenetic trees were inferred from the ITS, GDPH and EF1-α sequence data set using parsimony analysis with all characters weighted equally and all gaps treated as missing data.

Morphological studies

Conidia and conidiophores taken directly from nature hosts were examined in 3% KOH, using a Zeiss Axiophot 2 microscopy with Axiocam CCD camera and AxioVision digital imaging software (AxioVision Software Release 3.1.v.3–2002; Carl Zeiss Vision Imaging Systems). Cultural characteristics and morphology were determined on PDA, and water agar and wheat straw (WSA) [2 % water agar (10 g/L) with autoclaved wheat straw placed onto the medium] and incubated for 7–10 days at 25°C under 12/12 h alternation of near-UV light. 20 µL conidial suspension (10^4 conidia/mL) were dropped on the WA (water agar) medium at 25°C for 24 h and characteristics of conidia germination were observed.

Scanning electron microscopy

The 0.5 × 0.5 mm^2 pieces from diseased spots with conidia and conidiophores were placed in glutaraldehyde (2.5% vol/vol) for 48 h at 4°C. Samples were washed three times in 0.1 M sodium phosphate buffer (pH 7.0) and post-fixed in osmium tetroxide (1% wt/vol) for 1.5 h at 20°C. Samples were rinsed thoroughly with 0.2 M phosphate buffer (pH 6.8) and dehydrated in a graded ethanol series (30, 50, 70, 80, 90, 95, and 100%). Pieces were embedded in Spurr's epoxy resin. The sites containing conidia were identified by light microscopy and ultrathin sections were cut with a Reichert-Jung Ultracut E Ultramicrotome with a diamond knife. Ultrathin sections were collected on Formvar-coated slot copper grids; after drying, the grids were stained with uranyl acetate and lead citrate and examined using a transmission electron microscope (Hitachi S-3000N, Tokyo, Japan).

Pathogenicity Tests

Healthy field cuttings of *Z. latifolia* with 3–5 nodes were cultivated in a 25-cm diam. barrel with cultivated soil and placed in greenhouse at 26–28°C for one month. *Oryza sativa* seedlings grown in seedbeds were transplanted into a 20-cm diam. barrel and placed in greenhouse under the same conditions for one month.

An aqueous inoculation suspension (1×10^5 conidia/mL) with 1% vol/vol Tween-20 was prepared from 8–10-day old cultures. Pathogenicity tests were conducted on healthy leaves of *Zizania latifolia* and *Oryza sativa*. A 50 µL conidial suspension was dropped on two sides of each leaf and then inoculated plants were covered by large plastic bags and incubated at 27–28°C for 48 h after which the plastic bags were removed. Control leaves were inoculated with 50 µL sterile water. Symptoms and sizes of diseased spots were observed and recorded each day. The fungus was re-isolated by cutting small portions from the margin of diseased spots; these were surface sterilized and placed on PDA plates. The experiments were repeated twice to confirm the results.

Results

Phylogenetic analysis

Isolates of *Bipolaris* were obtained from *Z. latifolia* and purified, and six single-spore isolates were selected for study. Six strains of *Bipolaris* from *Z. latifolia* had identical ITS and GPDH gene sequences, but in the EF-1a gene sequence there was one base difference between the two strains from Hangzhou (MFLUCC13-0511, MFLUCC13-0512) and the other four strains (MFLUCC13-0513, MFLUCC13-0514, MFLUCC13-0515, MFLUCC13-0516). A GenBank search showed that there were no sequence data submitted for *B. zizaniae,* but a blast search of our ITS, GPDH, and EF1-α sequences from

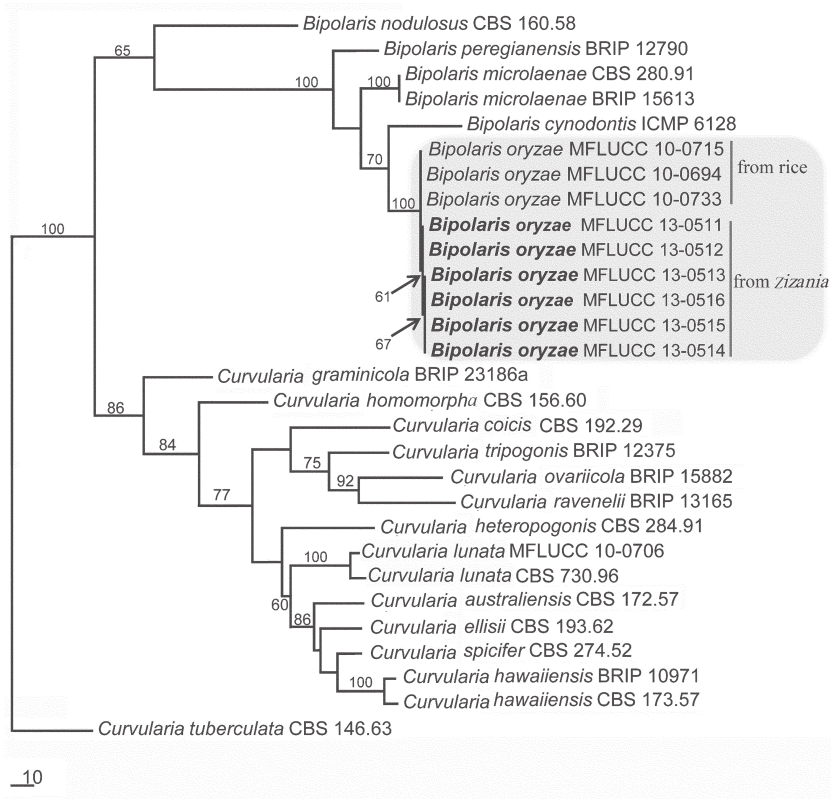

Figure 2. Phylogram generated from parsimony analysis based on ITS, GPDH and EF 1-α sequence data of *Bipolaris* and *Curvularia* species. Data were analysed with random sequence addition, unweighted parsimony and by treating gaps as missing data. Bootstrap values ≥50% are indicated. Species names are followed by the number of the deposited voucher. The tree is rooted with *Curvularia tuberculata.*

B. zizaniae showed a 99–100% similarity with those of *B. oryzae* lodged in GenBank. Of the 63 reliable or voucher *B. oryzae* ITS sequences in GenBank, 11 were identical with *B. zizaniae* and 41 differed by 1–2 bases. Similarly, of the 16 reliable or voucher *B. oryzae* GPDH sequences in GenBank, one was identical with *B. zizaniae* and 15 differed by 1–2 bases. Finally, of five *B. oryzae* EF1-α sequences (Manamgoda et al. 2012), four were identical with *B. zizaniae* and one differed by 1–2 bases.

A parsimony tree was constructed from the ITS, GPDH, and EF1-α data from 29 fungal isolates (Table 1). The four *Bipolaris* isolates from *Z. latifolia* with identical sequences grouped together with another two *Bipolaris* isolates from *Z. latifolia* (with one base difference) with 61% bootstrap value support (Fig. 2). This group clustered with three *B. oryzae* isolates with a 100% bootstrap support. Fig. 2 clearly shows that the *Bipolaris* isolates from *Z. latifolia* are inseparable from *B. oryzae*. Sequence variation between *Bipolaris* isolates from *Z. latifolia* and *B. oryzae* isolates was less than interspecific variation. Six *Bipolaris* isolates from *Z. latifolia* and three *B. oryzae* isolates clustered with *B. coffeana* Sivan. to form a clade with a 70% bootstrap value support (Fig. 2). However, the *B. coffeana* subclade (based on a single culture) and the *B. oryzae*/*B. zizaniae* subclade were separated by >40 base differences, indicating that they are not conspecific.

The description below is based on our collections of *Bipolaris* isolates from *Z. latifolia*. Except for size variation, these characters are almost identical to those from the type specimen from the same host from Japan (Nisikado 1929). The pathogen causes brown spots on leaves of *Zizania latifolia* and infects almost all aerial parts of the plant.

Bipolaris oryzae (Breda de Haan) Shoemaker, Can. J. Bot. 37: 883 (1959) Fig. 3

≡ *Helminthosporium oryzae* Breda de Haan, Bulletin Inst. Bot. Buitenzorg 6: 11 (1900)

= *Ophiobolus miyabeanus* S. Ito & Kurib., Ann. Phytopath. Soc. Japan 2(1): 7 (1927)

≡ *Cochliobolus miyabeanus* (S. Ito & Kurib.) Drechsler ex Dastur, Indian. J. Agr.Res. 12: 733 (1942)

= *Helminthosporium zizaniae* Y. Nisik., Ber. Ohara Inst. Landw. Forsch. Kurashiki 4: 122 (1929)

≡ *Bipolaris zizaniae* (Y. Nisik.) Shoemaker, Can. J. Bot. 37: 885 (1959)

Leaf spots dark brown with a paler centre 0.3–0.5 × 0.3–0.6 cm. On a natural host, conidiophores single or occasionally fasciculate in small groups, rarely branched, geniculate or straight in sterile part, then becoming slightly to distinctly geniculate, cicatrized with scars often inflated at base, medium olivaceous-brown below, paler at the apex, multiseptate, 106–395 μm long, swollen to 7–9 μm diam at base, then narrowing to 6–7 μm diam (middle)

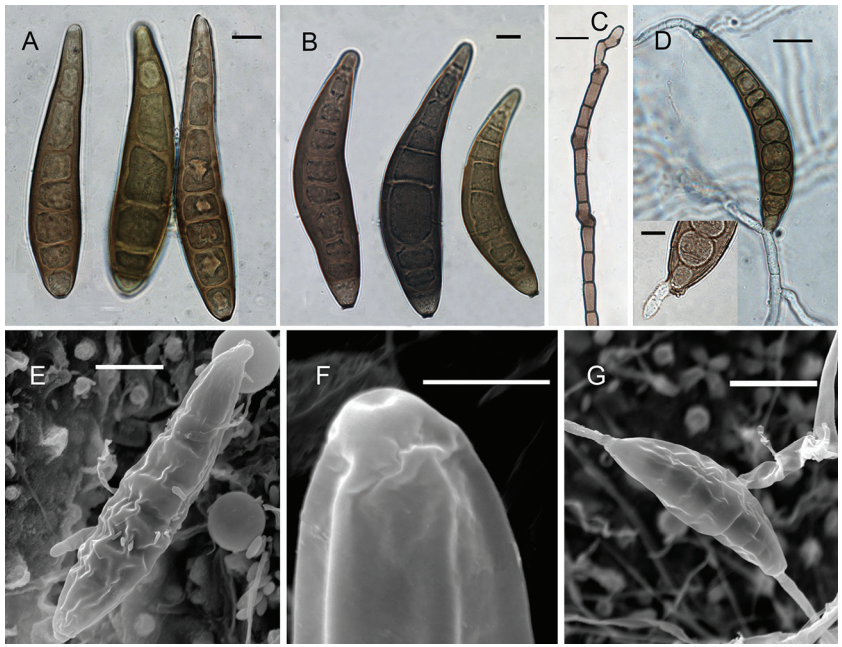

Figure 3. *Bipolaris oryzae* (isolates from Z. *latifolia*). A–D. Light microscope micrographs. A. Conidia. B. Conidia on WSA. C. Conidiophore. D. Germinated conidium with one germ tube at each end. Basal germ tube direction of growth (left below) is semiaxial, close to hilum. E–G. SEM micrographs. E. Conidia. F. Hilum. G. Germinated conidium with two polar germ tubes. Scale bars: A, B, D(lower) = 10 μm; C, D(upper) = 20 μm; E = 15 μm; F = 5 μm; G = 25 μm.

and 3–5 μm (apex). Conidia (65–)78–110(–131) × (15–)17–28(–34) μm, 6–10 distoseptate, olivaceous brown or yellowish brown, obclavate, navicular, straight or slightly curved, tapering towards the apex, hilum dark, conspicuous, sometimes slightly protruding. Conidia germinate by the production of two polar germ tubes. The sexual morph is not found in nature.

On WSA, conidiophores 212–646 × 5.5–10.5 μm, olivaceous-brown, paler at the apex. Conidia brown or dark brown, obclavate, fusoid, often curved, (73–)80–125(–130) × (1–)18–28(–31) μm, 6–11-distoseptate, hilum dark, conspicuously protruding or truncate.

Materials examined: **China**, **Zhejiang Province**, Hangzhou Suburbs, September 2012, Zilan Xiao (MFLU13-0092; cultures MFLUCC13-0511 = ZJU0101, MFLUCC13-0512 = ZJU0102); **Anhui Province**, Yuexi County, September 2012, Zilan Xiao (MFLU13-0093; cultures MFLUCC13-0513 = ZJU0201, MFLUCC13-0514 = ZJU0202); **Fujian Province**, Putian city, October 2012, Zilan Xiao (MFLU13-0094; cultures MFLUCC13-0515 = ZJU0301, MFLUCC13-0516 = ZJU0302).

Table 2. Synopsis of conidial size and number of distosepta in *Bipolaris oryzae* from *Zizania latifolia* from different origins.

	Isolate	Length (μm)	Width (μm)	Distosepta
On hosts	MFLU 13-0092	99 ± 11.5	25 ± 3.0	(5–)7–9
	MFLU 13-0093	87 ± 8.7	20 ± 3.6	(5–)7–10
	MFLU 13-0094	92 ± 10.6	23 ± 3.5	(5–)6–10
On WSA	MFLUCC 13-0511	96 ± 11	24 ± 3.8	6–9(–11)
	MFLUCC 13-0513	86 ± 7.8	19 ± 5.4	6–10
	MFLUCC 13-0515	90 ± 5.6	21 ± 6.7	6–10(–11)

Comments—Classification in the genus *Bipolaris* was previously based on morphology, with conidial characters being considered as important in species delimitation. In this study conidia of *Bipolaris* isolates from *Z. latifolia* are shorter (78–110 μm) and wider (17–28 μm) than those reported in the protologue (25–166 × 12–13 μm; Nisikado 1929). Although there is some variation in conidial size and distosepta in *Bipolaris* isolates from *Z. latifolia* from different locations in China (Table 2), the morphological differences are not significant. We found it impossible to distinguish *B. oryzae* from *B. zizaniae* based on morphological characters.

Morphology and molecular phylogeny confirm that *Bipolaris zizaniae* and *B. oryzae* are conspecific. *Bipolaris oryzae* is the correct name for this expanded taxon because the epithet *oryzae* (published in 1900) has priority over *zizaniae* (published in 1929). We follow Manamgoda et al. (2012, 2014) in preferring *Bipolaris* to the earlier generic name *Cochliobolus* (proposed for the sexual state).

Pathogenicity testing

The isolates of *Bipolaris* isolated from Z. *latifolia* infected the leaves of *Z. latifolia*. At 48 h after inoculation, *Bipolaris* isolates from *Z. latifolia* induced small flecks or expanded spots on the lower surface of *Z. latifolia* leaves. Symptoms were similar to those observed in the wild (Fig. 1e,f), and no disease was found on upper surface of inoculated or control leaves. Five days after inoculation, diseased spots increased in size (Fig. 1e). Symptoms of leaf blight occurred 18 days after inoculation (Fig. 1f). During the same period, disease symptoms did not develop on the upper surface or on control leaves.

Similarly, isolates of *Bipolaris* isolated from *Z. latifolia* also infected rice leaves. Small flecks occurred on the upper surface of inoculated leaves after five days and similar flecks occurred on the lower surface of leaves after six days. After 8–9 days, diseased spots increased more rapidly in size on the upper surface of leaves than on the lower surface of leaves (Fig. 1g, h). The diseased spots were approximately 0.5 cm in diameter 12 days after inoculation (Fig. 1i).

Discussion

Zizania latifolia, which originated in the northern region of China, was once used as an important grain in ancient times. Cultivated *Z. latifolia* was disseminated to Taihu Lake from north to south, and the Taihu Lake basin is the region associated with the origin of jiaobai (Xu et al. 2008). Jiaobai cultivation was introduced to eastern and southeastern Asia, including the Russian Far East, Japan, and Korea (Thrower & Chan 1980, Guo et al. 2007). It is possible that brown spot was also introduced to the jiaobai-growing areas with the planting material.

Zizania latifolia commonly grows in shallow water and can be found at the edges of lakes, ponds, wetlands, and flooded fields (Thrower & Chan 980, Guo et al. 2007). In some cases, *Z. latifolia* is able to grow in deeper water (Yamasaki & Tange1981), because it has a well-developed ventilation system (Yamasaki 1984, 1987). Thus, *Z. latifolia* growing conditions are similar to that of rice. In the jiaobai-growing areas, *Z. latifolia* is usually grown in paddy fields or in rotation with rice. The fact that pathogenic brown spot of *Z. latifolia* also infects rice may be a result of long-term evolution. In morphological taxonomy, phenotypic plasticity and homoplasy may influence fungal species delimitation (Pino-Bodas et al. 2011). *Bipolaris zizaniae* and *B. oryzae* have previously been delineated based on morphology, although species boundaries are not clear (Chang 1974; Tsuda & Ueyama 1975, 1976). *Bipolaris zizaniae* can be crossed with *B. oryzae* to form the sexual state, and both species can infect *Zizania* (Chang 1974, Tsuda et al. 1975). The data presented here and in previous studies suggest that *B. oryzae* and *B. zizaniae* are synonymous. Our gene sequence data analysis shows unequivocally that *B. oryzae* and *B. zizaniae* are conspecific.

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